Original Report:
Obesity

PPARG-LYPLAL1 Multi-Allelic Combination Associated with Obesity and Overweight in Mexican Adolescent Females

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Objective: We studied multi-loci variants to identify the contribution of six candidate genes (*ADIPOQ, CDH13, LYPLAL1, MC4R, PPARG* and *PGC1A*) in the development of obesity and overweight.

Design: We genotyped 404 chromosomes with eleven SNPs in Mexican female adolescents, who were subdivided into two groups (obesity-overweight and normal-weight) using the World Health Organization parameters. Genomic (800 chromosomes) and ancestral (208 chromosomes) controls were included to reduce the population bias. Anthropometric measurements, biochemical parameters, and caloric intake were obtained only in the groups of Mexican female adolescents.

Results: A positive genotype-phenotype association was found that involves the multi-allelic combination of three risk alleles (one in *PPARG* and two in *LYPLAL1*) with obesity and overweight (OR=3.1, P=.010). This combination also exhibited a significant association with waist circumference (P=.030) and triglycerides levels (P=.030). These associations were supported by a logistic regression analysis adjusted for several confounding variables.

Conclusions: Our data suggest the joint participation of *PPARG-LYPLAL1* genes in metabolic disorders development. Hence, these genes could act as potential biomarkers in obesity and overweight. Our findings underscore the complexity of metabolic disorders and provide evidence about the importance of multi-loci analysis to study complex diseases. *Ethn Dis*. 2016;26(4):477-484; doi:10.18865/ed.26.4.477.

Keywords: Multi-allelic Combination; *LYPLAL1; PPARG; ADIPOQ; MC4R; CDH13;* Obesity; Mexico

Introduction

Metabolic disorders (MD) are multifactorial conditions that encompass the most important public health problems worldwide.[1] Obesity and overweight are defined as excessive fat accumulation and chronic inflammation states with important consequences on health. In the last decades, the prevalence of MD have increased dramatically.[1] Thus, the medical cost related to them and their concomitant diseases (cardiovascular disease, diabetes and various cancers) is 10 times higher than in non-obese phenotypes.[2] This is particularly disturbing in the Mexican population where the prevalence of obesity and overweight in adults and children is 71% and 34%, respectively.[3] Hence, the knowledge about MD in childhood and adolescence is important given that risk factors in these age strata predict susceptibility for MD in adulthood.[4]

Genetic contribution is a keystone in the development of MD; yet, the study of genes separately does not explain multi-genic diseases. Thus, the identification of locus combinations could be the best model for predicting genetic association patterns.[5] Besides, the analysis of gene collections involved in diverse biological processes could exert a larger effect on the susceptibility of polygenic disorders and could become prognostic biomarkers.[5,6]

Genome-wide association studies (GWAS) have identified genetic markers with critical roles in lipid storage, adipocyte differentiation, inflammation, and satiety.[6] These markers have been explored mainly in European and Asian populations,

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where *PPARG, ADIPOQ, MC4R,* and *FTO* have been associated with MD.[7-11] Nevertheless, the contribution of these candidate genes remains almost unexplored in Mexico, which presents the worst problems in obesity and overweight worldwide.

Thus, our study aim was to evaluate the possible association of 11 SNPs in six candidate genes (*ADIPOQ, CDH13, LYPLAL1, MC4R, PPARG* and *PGC1A*) with obesity in Mexican adolescent females. All loci except *PGC1A* have been uncovered through GWAS related to metabolic conditions.

Our study aim was to evaluate the possible association of 11 SNPs in six candidate genes (ADIPOQ, CDH13, LYPLAL1, MC4R, PPARG and PGC1A) with obesity in Mexican adolescent females.

Materials and Methods

Study Population

We assessed 706 unrelated individuals born in Mexico, with three generations of ancestors born in this country, divided into four groups. The first group (obesity and overweight, n=99) and the second group (normal weight, n=103) consisted of female adolescents (average age: 17 years; range: 15-19) recruited at a high school in Mexico City. Obesity and overweight were defined by the anthropometric measurements of weight and height using calibrated instruments (balanced scale Terralion-Fitness coach premium 11328 and stadiometer SECA 208). Body mass index (BMI) was determined by dividing weight (in kilograms, kg) by the square of height (m^2). In agreement with the World Health Organization, we used the child growth standards to define overweight and obesity. Adolescents whose indicators of age and BMI were between the 15th-84.99th percentiles were classified as normal weight, from the 85th-96.9th were overweight, and higher than 97th percentile were considered obese. Adolescents with overweight and obesity were included in the same group (OW-OB). Waist circumference (WC) was also obtained using the midpoint between the lowest rib and the iliac crest. Caloric intake was assessed through a 24-hour dietary recall, performed by a nutritionist to obtain total caloric intake, carbohydrate, protein, and lipid percentages. Each participant answered a questionnaire of physical activity (type and time of physical activity per week). Metabolic equivalents (METs) were calculated for participants reporting any extra activity.[12]

Given that the Mexican population is sub-structured, spurious associations can occur if gene frequencies differ among subpopulations.[13,14] To diminish the bias caused by the recent admixture, we included a genomic control (GC).[13,15] GC included 400 individuals (200 females and 200 males) belonging to Mestizo population. GC is a control to estimate the variance within population because members of the Mestizo population have different ethnic backgrounds. This group was employed to determine gene frequencies, Hardy-Weinberg expectation (HWE), and linkage disequilibrium (LD). This was a sub-sample obtained from a representative group of 1640 unrelated individuals from the Central Valley of Mexico in which only the people whose eight great-grandparents were born in Mexico were eligible.[14]

Because the Amerindian ancestry is most prominent in the central region of Mexico, an ancestry control was included to clarify the differences in the gene frequencies.[16] This group consisted of 104 participants belonging to Mazahuas (n=21), Me'Phaas (n=32), and Nahuas (n=51) populations. (Table available upon request to corresponding author.)

Each participant signed an informed consent letter validated by the Ethics Committee of the Center for Research and Advanced Studies (CINVESTAV-IPN) in Mexico City and the National Institute of Perinatology "Isidro Espinoza de los Reyes." The informed consent was also signed by at least one parent; afterward, a blood sample was obtained.

Biochemical Analysis

Biochemical analyses were only performed in the first two groups. Venous blood samples (12- to 14-hour fasting) were obtained from each participant. Using these samples, glucose levels (glucose oxidase method), total serum cholesterol (mg/dL), HDL (mg/dL), and triglycerides (mg/dL) were determined using standardized protocols (Biosystems, Barcelona, Spain).

Molecular Analysis

DNA was extracted from peripheral blood leukocytes using a commercial

kit (Jena Bioscience, Jena, Germany). Eleven SNPs (*ADIPOQ*-rs864265 and rs1501299, *CDH13*-rs3865188, *LYPLAL1*-rs7552206, rs2820446 and rs2605100, *MC4R*-rs12970134, rs17700633 and rs17782313, *PPARG*-rs18801282, and *PGC1A*-rs8192678) were analysed using TaqMan assay (Applied Biosystems, Carlsbad, CA, USA). PCR cycling conditions consisted of pre-incubation period (10 min at 95ºC) followed by 40 cycles of 15" at 92ºC and 90" at 60ºC. The C1000 Touch Thermal Cycler (Bio-Rad, Richmond, CA) was employed for data acquisition; all experiments were performed by duplicate (r=.98. P≤.050).

Population Genetics Parameters

Genetic frequencies, HWE and LD between loci were estimated using Arlequin v 3.5 software.[17] Frequency of multi-allelic variants was estimated by direct counting.

Statistical Analysis

To evaluate the potential differences between groups, different tests (t-test, Wilcoxon rank-sum or chi square) were carried out depending on the variable distributions. Differences in genetic frequencies were analyzed by chi square test using the STATA 12.0 software package (Stata Corp., College Station, TX).

Continuous variables (caloric intake, physical activity, waist circumference and triglycerides levels) were categorized for logistic regression models as follows: caloric intake and physical activity were divided by terciles. To caloric intake the subdivisions were low (1070-1454.9), medium (1455-1759) and high (1760). To physical activity the terciles were low (0<X≤348), medium (349-524.9) and high (>525). WC and triglycerides levels were classified as high if the measures exceeded 80 cm and 130 mg/dL, respectively.

Table 1. Descriptive characteristics of normal-weight and overweight-obese female adolescents

Characteristics	Overweight-obese, n=99	Normal-weight, n=103	P
Age, years			
Mean ± SD	16.60 ± 1.03	16.50 ± .98	.230
P_{10}-P_{90}	15.00-18.00	15.00-18.00	
Anthropometrics			
Height, cm			
Mean ± SD	157.30±6.63	156.54 ± 5.91	.380
P_{10}-P_{90}	150.00-163.30	149.00-165.00	
Weight (kg)			
Mean ± SD	67.14 ± 9.90	51.84 ± 5.89	<.001
P_{10}-P_{90}	57.50-82.80	45.00-60.16	
BMI			
Mean ± SD	26.60 ± 3.45	20.50 ± 1.85	<.001
P_{10}-P_{90}	23.00-32.00	18.00-23.00	
Waist circumference, cm			
Mean ± SD	82.50 ± 8.84	70.57 ± 5.54	<.001
P_{10}-P_{90}	73.00-94.90	63.32-77.92	
Metabolic parameters			
Cholesterol, mg/dL			
Mean ± SD	168.24 ± 31.85	155.94 ± 29.18	.003
P_{10}-P_{90}	128.40-206.60	119.00-192.80	
Triglycerides, mg/dL			
Mean ± SD	124.41 ± 67.11	93.82 ± 38.69	<.001
P_{10}-P_{90}	68.10-187.20	57.40-146.60	
Glucose, mg/dL			
Mean ± SD	93.31 ± 9.00	91.57 ± 18.17	.090
P_{10}-P_{90}	80.00-106.00	81.00-102.00	
24-hours dietary recall			
Caloric intake, kcal/d			
Median	1356.0	1455.0	.760
P_{10}-P_{90}	895.0-2390.0	734.4-2278.2	
Carbohydrates, %			
Median	49.8	49.7	.560
P_{10}-P_{90}	37.1-64.0	37.7-63.2	
Proteins, %			
Median	17.0	16.6	.180
P_{10}-P_{90}	12.2-24.2	11.3-22.6	
Lipids, %			
Median	32.0	32.6	.740
P_{10}-P_{90}	17.0-41.5	19.5-42.8	
Physical activity, METs/week			
None (%)	35 (35.3)	46 (44.7)	
Low (%)	24 (24.2)	16 (15.5)	.370
Medium (%)	22 (22.2)	21 (20.4)	
High (%)	18 (18.2)	20 (19.4)	

P for t-test, Wilcoxon rank sum test or chi square test.

Comparison with Other Populations

Our data were compared with 18 populations (n=12663), thus geno-

type information was collected from previous reports (Table available from corresponding author).[18] Population subdivision was assessed by analysis of molecular variance (AMOVA) using ethnicity as subdivision criterion with Arlequin v.3.5 software.[17]

Results

Anthropometric and Biochemical Measurements

The comparison between OW-OB and NW groups is shown in Table 1. Anthropometric parameters (weight, BMI, and WC) were higher in OW-OB than in NW (P≤.001). Also, significant differences were observed for total cholesterol (P≤.003) and plasma triglycerides (P≤.001).

Genetic Statistical Descriptive Analysis

Genetic frequency distributions for all loci in NW and OW-OB groups were calculated. (Table is available from corresponding author.) All polymorphisms studied exhibited similar gene distributions in all populations. No deviations in HWE and LD were found (Bonferroni's correction, P≤.004).

Genes Involved in Adipogenesis: PPARG and PGC1A

The allele C (>70%) and their homozygote states (>50%) were the most prominent in OW-OB as well in NW. PPARG-rs18801282-G, reported as risk allele, was more frequent in the OW-OB group than in NW (.17 vs .13), suggesting a possible association with overweight and obesity. No significant differences were found between groups in these polymorphisms.

Genes Involved in Inflammation: ADIPOQ and CDH13

The most frequent alleles were G (rs864265 and rs1501299) in ADIPOQ and A in CDH13. The homozygote state of these alleles was also the most prominent. No significant differences were found between OW-OB and NW.

Genes Involved in Lipid Storage: LYPLAL1

We evaluated three polymorphisms in *LYPLAL1*: rs7552206, rs2820446, and rs2605100. None of them showed significant differences between groups. In the particular case of rs7552206-AA genotype, this was slightly more frequent in the OW-OB group.

Overeating Disorders: MC4R

Regarding MC4R-rs12970134, rs17700633, and rs17782313, OW-OB and NW groups showed similar distributions; therefore, no statistical differences were found.

Comparisons with Genome Control

Following the STrengthening the REporting of Genetic Association Studies statement, we included a GC and ancestry control groups.[13] These were composed of the most important ethnic groups in Mexico (Mestizos and Amerindians) in which genetic statistical descriptive analyses were carried out.

Concerning HWE, locus LYPLAL1-rs2820446 exhibited an important departure in the Mestizo group (P<.001) owing to homozygous excess (F_{is}=.350) in both alleles. Interestingly, the GG genotype was the most frequent in Amerindian populations (range 28% to 51%).

Regarding LD, the SNPs studied in LYPLAL1 and MC4R loci showed important LD in Mestizo and Amerindian populations (P<.001). Additionally, a nuance LD (P<.050) in ADIPOQ and PGC1A was found in Mazahuas and GC populations. Similar patterns were also found between CDH13-ADIPOQ (Nahuas), CDH13-MC4R (NW), CDH13-LYPLAL1 (OB-OW) and CDH13-PPARG (NW) as well between PGC1A-MC4R (NW), PGC1A-LYPLAL1 (OW-OB, Nahuas), PGC1A-ADIPOQ (GC). ADIPOQ also exhibited a feeble LD (P<0.050) with PPARG, LYPLAL1 and within ADIPOQ SNPs in GC population and Me'Phaas (ADIPOQ-LYPLAL1). Given that this LD was found in separated genes, LD patterns were detected in other populations. Thus, we detected the LD patterns in Chinese Han in Beijing, China (CHB), Utah residents with Northern and Western European ancestry from the CEPH collection (CEU) and Yoruba in Ibadan, Nigeria (YRI) from HapMap project (https://hapmap.ncbi.nlm.nih.gov/). Different patterns were also found among these populations. LYPLAL1 strong LD (P<.001) was found in CEU, whereas CHB and YRI only presented subtle LD (P<.050). Rather, a strong LD was found in MC4R (rs12970134-rs17782313) in CHB and CEU. Interestingly, YRI population showed similar patterns of LD in PGC1A-LYPLAL1 such as OW-OB and Nahua populations.

Ultimately, important differences were found between OW-OB and NW

in all pairs of loci: CDH13-MC4R-rs12970134 (P=0.006, OR=3.8, 95%CI=1.4-10.9), CDH13-MC4R-rs17700633 (P=.047, OR=3.5, 95% CI=.9-12.9), and CDH13-MC4R-rs17782313 (P=1.2 x 10^{-5}, OR=2.26, 95% CI=3.0-17.4). These differences were lost when comparing OW-OB and GC, suggesting a spurious association.

Multidimensional Scale Analysis

To detect any differences between OW-OB and NW, a multidimensional scale (MDS) analysis was performed. OW-OB appeared separated from NW in the plot, which were supported by inclusion of GC, suggesting genetic differences among the groups (Figure 1, Stress value<.001).

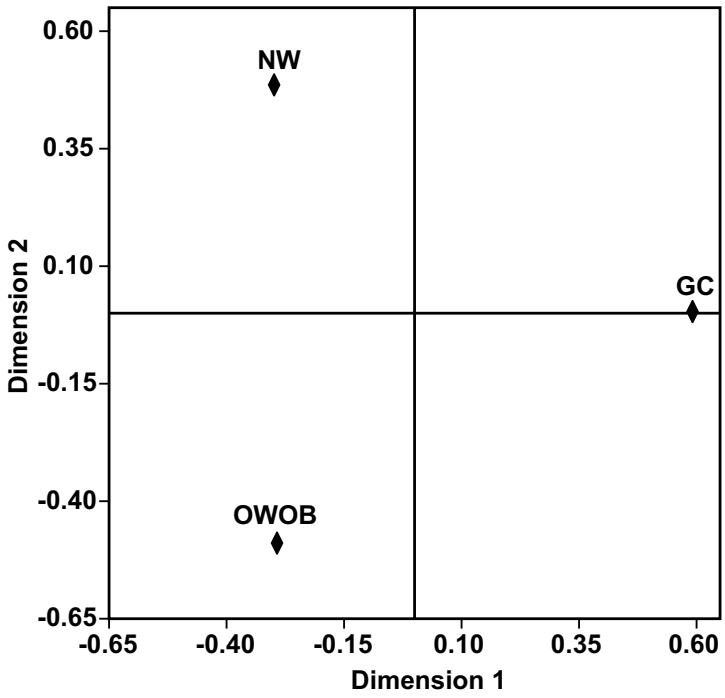

Figure 1. Multidimensional Scale Analysis showing the genetic differences among overweight-obesity, normal weight, and genomic control.
GC = Genomic control, NW = Normal weight, OW-OB = Overweight-obesity.

Multi-allelic Analysis

Given that obesity and overweight are complex conditions, multi-allelic variants at different loci using the risk alleles previously reported were evaluated. PPARG, ADIPOQ, CDH13, and LYPLAL1 showed important differences. From these loci, four different multi-allelic combinations (MAC) exhibited possible relations with obesity and overweight. These MACs were recognised as follows: MAC-1 (PPARG-rs1801282-G-LYPLAL1-rs2820446-G), MAC-2 (PPARG-rs1801282-G-LYPLAL1-rs2820446-G-rs7552206-A), MAC-3 (PPARG-rs1801282-G-ADIPOQ-rs864265-T-LYPLAL1-rs7552206-A), and MAC4 (CDH13-rs3865188-T-LYPLAL1-rs2820446-G-rs7552206-A).

Table 2 shows the differences among groups using MAC; all data were adjusted for confounder variables through logistic regression analysis. A marginal difference between NW and OW-OB (P=.080, OR=1.8, 95%CI=0.92-3.85) was found in MAC-1. MAC-2 presented stronger differences between OW-OB and NW (P=0.010, OR=3.1, 95%CI=1.27-7.62), which were maintained to compare with the whole GC group (P=0.002, OR=2.64, 95%CI=1.43-4.88). These findings were confirmed by different statistical analyses (chi square test, P =0.017 and exact test, P =0.014). Rather, MAC-3 and MAC-4 do not exhibited significant differences.

MAC-2 vs Anthropometric and Biochemical Parameters

Having found these differences, a possible contribution of MAC-2 with anthropometric and biochemical parameters was considered. Logistic regression analysis showed that MAC-2 could be related to WC (P=.034) and triglyceride levels (P=.031), suggesting a possible contribution of PPARG and LYPLAL1 to obesity and overweight. Results of the logistic regression analysis were adjusted for confounder variables supporting our findings (Table available from corresponding author).

Comparison among Populations

Ultimately, we compared the frequency of MAC-2 in different populations using 1000 genome data.[18] Overall, African, Asian, and European populations showed low frequencies of MAC-2 (≤ 6%), of which Africans (.15%) and Asians (2.5%) presented the lowest percentages.[18] Regarding

Table 2. Comparative statistics for multi-allelic analyses among normal-weight, overweight-obese and genomic control

Multi-Allelic Combination	Overweight-obese vs normal-weight			Overweight-obese vs GC women			Overweight-obese vs GC all		
	%	OR (95% CI)	P[a]	%	OR (95% CI)	P[a]	%	OR (95% CI)	P[a]
MAC-1	25.2/16.5	1.88 (.92-3.85)	.080	25.2/17.0	1.65 (.92-2.96)	.09	25.2/11.2	2.66 (1.54- 4.62)	.001
MAC-2	19.2/7.8	3.1 (1.27-7.62)	.010	19.2/12.0	1.74 (.90-3.36)	.09	19.2/8.2	2.64 (1.43-4.88)	.002
MAC-3	13.1/5.8	2.65 (.94-7.48)	.060	13.1/8.0	1.74 (.80-3.77)	.16	13.1/5.5	2.60 (1.26-5.36)	.010
MAC-4	23.2/16.5	1.40 (.68-2.87)	.350	23.2/25.5	.88 (.50-1.55)	.67	23.2/18.7	1.31 (.77-2.23)	.320

a. Adjusted by: age, caloric intake and physical activity.
MAC-1: *PPARG*-rs1801282-G, *LYPLAL1*-rs2820446-G; MAC-2: *PPARG*-rs1801282-G, *LYPLAL1*-rs2820446-G, rs7552206-A; MAC-3: *PPARG*-rs1801282-G, *ADIPOQ*-rs864265-T, *LYPLAL1*-rs7552206-A; MAC-4: *CDH13*-rs3865188-T, *LYPLAL1*- rs2820446-G, rs7552206-A.

NW and GC, these also exhibited low frequencies of MAC-2 (~8%). Strikingly, the OW-OB group showed twice the frequency of MAC-2 (19%) than NW. This tendency was also observed in Mexican ancestry in Los Angeles, California (MXL), which presented percentages higher than 10%.[18] Of note, Amerindian populations exhibited the highest frequencies of MAC-2 (>25%), even higher than the OW-OB group. Me'Phaas exhibited the lowest frequency of this MAC (<1%).

Discussion

Herein, we assessed 11 polymorphisms related to adipogenesis, inflammation, lipid storage, and satiety to search for genetic risk factors to obesity and overweight in a sample of Mexican adolescent females. The study of genes separately exhibited marginal associations. However, the multi-allelic combination *PPARG-LYPLAL1* suggested a positive association with WC and triglyceride levels. Thus, individuals carrying three risk alleles increased three-fold the risk of these MD.

Our findings were in line with prior studies where polymorphisms located on *LYPLAL1* were related to waist-hip ratio (WHR) and lipid metabolism.[6] One of these polymorphisms (rs2605100) was associated with type 2 diabetes, central obesity, and anthropometric traits.[19] This SNP is co-inherited with the SNPs included in MAC-2 (rs7552206 and rs2820446), supporting our findings.

Concerning *PPARG*, allelic variants on this locus have also been related to WHR.[20] Of note is the participation of the Ala12 allele, which has been related to dyslipidemias caused by low transcriptional activity.[2] Interestingly, Ala12 was subtly increased in the OW-OB group (17%) compared to NW (13%). This finding was previously described in insulin-resistance and obese women, reinforcing our results.[22] Interestingly, Amerindian populations (Mazahuas and Nahuas) exhibited the highest frequencies of the Ala12 allele (25%), which was in agreement with previous studies in Yaquis, Mayas, and Triquis.[23] Conversely, European (≤10%) and African (≤1%) ethnicities showed lower frequencies of Ala12 than in Mestizos and Amerindians.[18] Therefore, it is likely that Amerindian lineages could contribute to the development of MD. Preliminary research reported that lipid disorders (cholesterol and triglycerides) are more frequent in Amerindian-derived populations than in Europeans, justifying our results.[24] However, further studies are required to verify the association across different ethnicities.

Although our results were in agreement with previous observations, the collective participation of *PPARG-LYPLAL1* genes has been poorly studied in this context. One possible interaction pathway between them could be energy balance, where both genes play a critical role.[23, 25] It is possible that this interaction requires the participation of other molecular mediators (transcription factors) since recent research has suggested that *LYPLAL1* is not directly regulated by *PPARG*.[25] Another possibility could be that *LYPLAL1* may act as other lipases (lysophospholipase I) modulating the biological activity of different proteins, which in turn are regulated by *PPARG*.[26] Moreover, different hormones regulated by *PPARG* could provoke a differential expression of *LYPLAL1* by modifying its activity.[27]

Regarding the other MAC, our

results showed critical differences between groups that were maintained when compared with GC. These findings were confirmed by MDS suggesting important genetic differences (Figure 1). Previous research supports our data concerning the interaction between *PPARG-ADIPOQ*, suggesting an epistatic effect between them.[8,23,28]

In connection with LD presented in the different populations, two landscapes were found. The first one (*LYPLAL1* and *MC4R*) shows a real LD related to genetic distances (>1cM). This LD has been reported in prior studies and it is consistent with HapMap populations.[5,18,29] The second scenario depicted different patterns of LD in Amerindian and Mestizo populations. This LD is a spurious LD related to population history and demographic events.[30] Regarding Me'Phaas, LD was associated with inbreeding practices (*Fis*=0.210) given that it is an isolated population, thus they are identical by descent.[31] For the rest of the populations, LD could be the result of a recent admixture with Mestizo population.[32] Both populations (Amerindian and Mestizo) have different gene frequencies provoking an admixture LD (ALD). ALD will disappear through time because only genes that are very close and those combinations under selection maintain LD for a great number of generations. Consequently, these patterns were not replicated in HapMap populations reinforcing that LD in separated genes was spurious. Hence, the knowledge of LD patterns in the populations studied as well as in the ethnicities represented by these populations is a powerful tool in order to avoid spurious associations.

Even though our findings have been replicated in different ethnicities, it is worth mentioning that our results also differed from previous studies in Asian, European, and American populations.[29,33] The absence of replication may be associated with lower coverage of genetic markers in the replication samples as well as different sample sizes.[13] In this vein, our study comes with limitations owing to the small number of participants studied. Nonetheless, the GC, as well as the study of parental populations, lends robustness to our results.[34] These false associations are more prominent in admixed populations (such as the Mexican population), where the genetic association may reflect the genetic architecture of the population (ethnic dissimilarities) rather than the association of genes with the disease.[13] Our results should be interpreted with caution and as preliminary evidence, which requires further studies in large study populations to confirm these findings.

Conclusion

Our multi-allelic analysis supports the influence of genetic factors in obesity and overweight, suggesting that *PPARG-LYPLAL1* could act as potential biomarkers in cardiometabolic diseases. Our findings underscore the complexity of metabolic disorders and provide evidence about the importance of studying network models of genes. Further analyses are needed to pinpoint the interactive effects of different genes in complex diseases, as well as to elucidate the physiological role of *LYPLAL1*, which is still unknown. To our knowledge, this is the first research from Mexico where the joint relevance of genes has been studied.

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Conflict of Interest

No conflicts of interest to report.

Author Contributions

Research concept and design: Arenas-Sordo, Gómez; Acquisition of data: Hernández-Tobías, Noris, Santana, Reyna Samano, Arellano-Galindo, Arenas-Sordo, Rodríguez-Ventura, Gómez; Data analysis and interpretation: Hernández-Tobías, Torres-Sánchez, Reyna Samano, Brooks, Rodríguez-Ventura, Meraz-Ríos, Gómez; Manuscript draft: Hernández-Tobías, Noris, Santana, Torres-Sánchez, Brooks, Rodríguez-Ventura, Meraz-Ríos, Gómez; Statistical expertise: Torres-Sánchez, Gómez; Acquisition of funding: Gómez; Supervision: Noris, Santana, Arenas-Sordo, Meraz-Ríos, Gómez; Administrative: Hernández-Tobías, Noris, Santana, Reyna Samano, Arellano-Galindo, Brooks, Rodríguez-Ventura, Gómez

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